Volume 17 Number 11 1989 Nucleic Acids Research

The stereoselective enzymatic synthesis of 9-β-D-2'-deoxyribofuranosyl 1-deazapurine

D.Betbeder, D.W.Hutchinson and A.O'L.Richards

Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

Received March 22, 1989; Revised and Accepted April 28, 1989

ABSTRACT

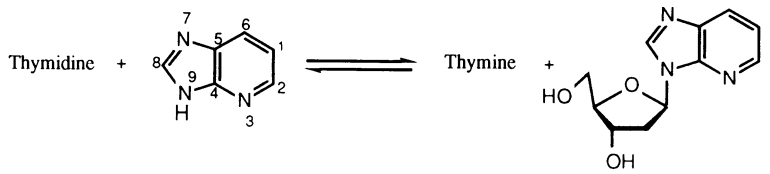
The transfer of 2-deoxyribose from thymidine to 1-deazapurine which is catalysed by N-deoxyribosyl transferases from *Lactobacillus leichmanii* occurs in high yield. This is a very stereoselective process and only one product, 9-β-D-2'-deoxyribofuranosyl 1-deazapurine, is formed. Nmr spectroscopy, and in particular, nuclear Overhauser enhancement experiments, confirm that the 2-deoxyribose moiety is bound to N-9 rather than N-7 and that the glycosidic link has the β-configuration.

INTRODUCTION

The importance of nucleoside analogues as antiviral and anticancer agents has encouraged the development of methods for their synthesis. We and others [1-3] have been investigating the use of nucleoside deoxyribosyl transferases (N-deoxyribosyl transferases, EC 2.4.2.6) from *Lactobacilli* and other organisms for the synthesis of nucleoside analogues to take advantage of the stereochemical control available in enzymatic reactions. Furthermore, as protecting groups are not normally required in enzymatic syntheses preparative procedures are simplified.

Little effort has been expended by previous workers on the structural proof of the products of these transfer reactions and in some cases [1] identification has only been by chromatographic comparison and no yields are given [3]. We wish to report the transfer of 2-deoxyribose from thymidine to 1-deazapurine (1*H*-imidazo[4,5-b]-pyridine) (1) in a convenient synthetic procedure which results in the formation in high yield of only one nucleoside, 9-β-D-2'-deoxyribofuranosyl 1-deazapurine (2). ^{1}H and ^{13}C nmr spectroscopy including nuclear Overhauser enhancement studies together with mass spectrometric evidence have been used to prove the structure of this compound.

In our synthetic work, a crude preparation of deoxyribosyl transferases from



(1) (2)

Lactobacillus leichmanii was used. This preparation contains, contrary to previously published observations [1, 4, 5], three transferase activities as resolved by ion-exchange FPLC [6]. The three transferases catalyse the following reaction:

$$\text{Base(1)-dRib} + \text{Base(2)} \rightleftharpoons \text{Base(2)-dRib} + \text{Base(1)}$$

where dRib = β-D-2-deoxyribofuranosyl-1

but have differing specifications for base residues. We find that the use of the crude transferase preparation rather than purified transferases enables the transfer of deoxyribose residues between a variety of bases to be more readily accomplished than when the individual enzymes are used.

MATERIALS AND METHODS

Thymidine and 1-deazapurine (4-azabenzimidazole) were commercially available from Aldrich Chemical Company Ltd. ^1H nmr spectra were run at 400 MHz and proton-decoupled ^{13}C nmr spectra at 100.62 MHz in solution in CD_3OD. Chemical shifts are given in ppm relative to TMS as internal standard. The high resolution ammonia chemical ionisation (CI) mass spectrum was recorded on a Kratos MS80 spectrometer.

Preparation of transferases

Lactobacillus leichmanii (ATCC 4797) were grown as described by Beck and Levin [7]. The cells were lysed with lysozyme (5 mg/ml) and mutanolysin (30 μg/ml) [8] and then passed through a French press at 10 000 psi keeping the temperature below 10°C. A crude preparation of the three transferases was obtained as the 35-70% ammonium sulphate precipitate of a heat-treated, cell-free extract. The precipitate was resuspended then dialysed twice against 0.02 M PIPES buffer pH 6.5 and the precipitate removed by filtration through a 0.45 μm ultrafilter (Millipore UK Ltd). The preparation obtained could be stored at -20 °C for up to three months without substantial loss of activity.

Deoxyribosyl transfer: synthesis of 9-β-D-2'-deoxyribofuranosyl 1-deazapurine

To 1-deazapurine (40 mg, 0.335 mmole) and thymidine (325 mg, 1.34 mmole) in

citrate buffer (200 ml, 0.25 mM pH 6) at 40°C was added crude transferase prepared as above (2 ml, 5 mg/ml, 4.5 U). After 3 h, reverse phase HPLC (Technopak 10 C18 column, elution with a gradient of 15-30% methanol/water) showed that all starting material had been consumed and that deoxyribose transfer was complete. The reaction mixture was lyophilised and the residue extracted with dichloromethane/methanol (9:1 v/v, 10 ml). The organic phase was applied to preparative silica tlc plates which were developed with dichloromethane/methanol (4:1 v/v) to yield 9-β-D-2-deoxyribofuranosyl 1-deazapurine (2) (60 mg, 76%). Ammonia CI mass spectrum: $(M + H)^+$ m/z 236.1032, $(C_{11}H_{13}N_3O_3 + H)^+$ requires m/z = 236.1036.
UV (MeOH) max 281 (3,980), 249.5 (2,590), 209 (4,820) nm.

Nmr data

^1H nmr: 8.71 (1Hs) H-8, 8.42 (1Hdd, J_{2-6} = 1.4, J_{1-2} = 4.9 Hz) H-2, 8.15 (1Hdd, J_{1-6} = 8.1, J_{6-2} = 1.4 Hz) H-6, 7.41 (1Hdd, J_{1-2} = 4.9, J_{1-6} = 8.1 Hz) H-1, 6.62 (1Hdd, $J_{1'-2'a}$ = 6.1, $J_{1'-2'b}$ = 8 Hz) H-1', 4.65 (1Hm) H-3', 4.13 (1Hm) H-4', 3.86 (2Hm) Hab-5', 2.93 (1Hm) H-2'a, 2.48 (1Hm) H-2'b.
^{13}C nmr: 145.26, 145.13, 137.09, 129.15, 124.68, 120.18, 89.77, 87.03, 73.07, 63.66, 41.36.

Nuclear Overhauser enhancement experiments

Irradiation of the signal at 6.63 ppm (H-1') caused enhancement of signals due to H-8, H-4' and H-2'b. Irradiation of the signal at 8.16 ppm (H-6) caused enhancement of the signal due to H-1 only.

Antiviral activity

The anti-herpes activity of 9-β-D-2'-deoxyribofuranosyl 1-deazapurine was tested by a 2 day plaque-reduction assay after the method of Collins and Bauer [9] using HSV-1 KOS strain and HSV-2 186 strain in Vero cells. No antiviral activity was observed.

RESULTS AND DISCUSSION

Only one 2'-deoxyribonucleoside was formed enzymatically from 1-deazapurine under our conditions using a mixture of deoxyribosyl transferases from *L leichmanii* even though there are three possible nitrogen atoms in 1-deazapurine to which the 2-deoxyribose residue might be bound. The 400 MHz ^1H nmr spectrum of the nucleoside (Figure 1a) indicated that this was 9-β-D-2'-deoxyribofuranosyl 1-deazapurine. Nuclear Overhauser enhancement experiments unequivocally showed that the nucleoside had the β-configuration and that the deoxyribose residue was attached to N-9. Irradiation of the signal due to H-1' at 6.63 ppm caused the enhancement of signals due to H-8, H-4'

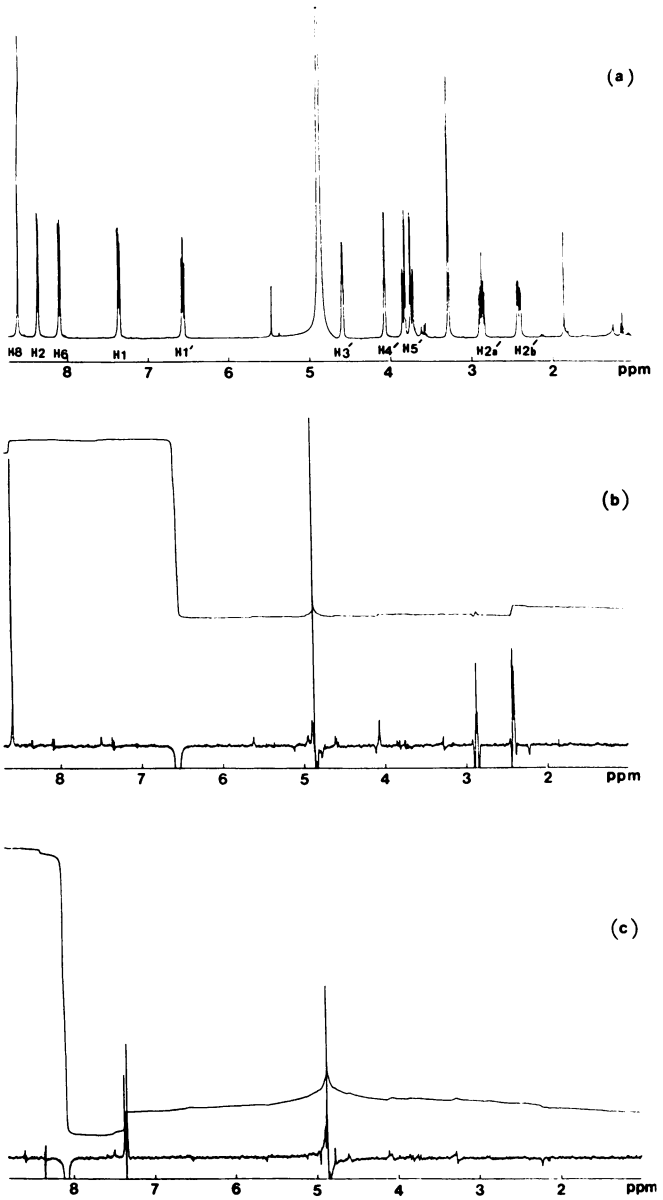


Figure 1 Nuclear Overhauser Effect Experiments
a 400 MHz ^{1}H nmr spectrum of 9-β-D-2'-deoxyribofuranosyl 1-deazapurine.
b Effect of irradiating signal at 6.63 ppm.
c Effect of irradiating signal at 8.16 ppm.

and the proton at H-2' on the underside of the deoxyribose ring (Figure 1b). This confirms the β-configuration of the glycosidic link. Irradiation of the signal due to H-6 at 8.16 ppm caused enhancement of only one signal, that due to H-1 the adjacent proton in the six-membered ring of the heterocycle (Figure 1c). This demonstrates that H-6 is not adjacent to the top (β) side of the deoxyribose ring and hence the sugar is attached to N-9. There is no evidence in the nmr spectrum of a signal due to the α-anomer and hence the transfer of deoxyribose to 1-deazapurine gives the β-anomer with greater than 99% stereospecificity. While nmr data on nucleosides synthesised enzymatically have been reported [2], this is the first time that the structure of a nucleoside product has been proved unequivocally by this means.

The synthesis of the new nucleoside occurred in virtually quantitative yield in 3 h. The new deoxynucleoside was isolated by simple chromatographic procedures which are a marked improvement on those previously reported for the enzymatic synthesis of deoxynucleosides on a semi-preparative scale [2]. This contrasts with the chemical syntheses of similar nucleosides, eg, 9-β-D-ribofuranosyl 1-deaza-adenosine has been obtained [10,11] after multistage preparations giving low overall yields of product and no synthesis of compound (2) has been published. 3-Deazapurine (^{1}H-imidazo[4,5-c] pyridine) is not an acceptor of deoxyribose using the transferases from *L leichmanii* under our reaction conditions when 1-deazapurine and other purines, eg, adenine or hypoxanthine are good acceptors. The lack of reactivity of 3-deazapurine may be because there is no nitrogen atom in position-3 in the six-membered ring which may be required to form a hydrogen bond in the active site(s) of the transferases. On the other hand, transfer of 2-deoxyribose residues to benzimidazoles has been achieved [12].

9-β-D-2'-Deoxyribofuranosyl 1-deazapurine did not inhibit the replication of either HSV-1 or HSV-2 as determined by the assay of Collins and Bauer [8]. Investigation of other biological properties of this nucleoside are in progress.

ACKNOWLEDGEMENTS

We wish to thank Dr O W Howarth for assistance with the nmr experiments and Dr J M Cameron of Glaxo Group Research for the studies on herpes virus replication. Financial support by the Medical Research Council is gratefully acknowledged.

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